IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

APPLICANT(S):	John Reynolds
APPLICATION NO.:	10/009,389
FILING DATE:	20 July 2002
TITLE:	Transformation of *Allium sp.* with *Agrobacterium* Using Embryogenic Callus Cultures
EXAMINER:	Georgia L. Helmer
GROUP ART UNIT:	1638
ATTY. DKT. NO.:	34703/0042

COMMISSIONER FOR PATENTS
P.O. BOX 1450
Alexandria, VA 22313-1450

37 C.F.R. § 1.131 DECLARATION OF PRIOR INVENTION

I, John Reynolds, hereby declare:

that I am the inventor named of the subject matter claimed in the above-identified patent application:

that I have reviewed the Office Action dated November 3, 2005, rejecting the patent application;

that I have reviewed the prior art references cited therein;

that the reference Eady, C.C., Transformation of Onion, 1998 Proceedings National Research Conference, Sacramento, CA, USA, was published in accordance with a conference that occurred between December 10 to December 12, 1998;

that the reference Eady, *et al.*, A comparison of four selective agents to use with *Allium cepa* immature embryos and immature embryo-derived cultures, Plant Cell Reports (1998) 18:117-121, published in the 1998 November issue of that publication;

that I have reviewed my records relating to the development of the subject matter claimed in the application, namely, methods for the transformation of *Allium sp.* with *Agrobacterium* using embryogenic callus cultures;

that subject matter reflected in the claims of the above patent application was invented by me, as reflected in my records, at least as early as October 1, 1998;

that the attached photocopies are from an internal Biotech Quarterly Report from Seminis Vegetable Seeds, Inc., from the 1st quarter of 1998 (which was produced by me prior to October 1, 1998), which has been redacted to remove confidential information not necessary to understanding the development of transformed *Allium cepa;*

that the Biotech Quarterly Report discloses results of experiments showing the development of transformed *Allium cepa* with a construct having as the DNA of interest a GUS reporter gene; and

that I, being warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon, declares that all statements made of the my own knowledge are true and that all statements made on information and belief are believed to be true.

Dated: 4.1.2005

By: John Reynolds, Inventor

Seminis Vegetable Seeds, Inc.

BIOTECH QUARTERLY REPORT

Confidential Information

Distribution outside the company in whole or in part requires written permission of the Author and Research Management.

Biotechnology Quarterly Report for the:	1st, Quarter of 1998
Submitted by :	John F. Reynolds, Maggie Garduno
I have copied to:	David Tricoli
Please copy to:	David Webster

Seminis Vegetable Seeds • 37437 Highway 16 • Woodland, California • Tel: (916) 666-0931 • Fax: (916) 666-5759

THIS PAGE BLANK (USPTO)

Crop(s)Onion
Program GMO

Significant Developments:

Can this information be broadly distributed throughout SVS? ***Yes***

- We observed GUS positive tissue after an Agrobacterium based transformation.

Primary Activities:

Can this information be broadly distributed throughout SVS? ***Yes***

We initially looked at GUS staining of our haploid embryo originating callus tissue. We tested three callus lines for background staining and found only minor reaction in one line.

We initiated an Agro. based transformation looking at 7 callus lines and 2 bacteria backgrounds, EHA105 and ABI with the GUS reporting gene. We observed staining in two of the callus lines using the EHA105 background. No staining was seen in the ABI or control callus. The staining pattern was typical for a transient/stable event using Agrobacterium. We did not see a light diffuse staining as observed in some control callus lines.

We stained the above Agro. transformed tissue at 4 days post induction, however, we were advised by . that best reaction time should be tested in the monocot systems. We examined staining 1,2, and 3, days post induction and found similar results at each day. We also looked at two genotypes in this test an saw staining in only one. The rate of staining was 10-20% of the callus pieces with up to 20 blue spots per callus piece.

In the above test we also examined plus and minus sonication and vacuum. There was no increase in the plus sonication/vacuum over the control. We expanded the study by looking at several sonication times using a different callus line. Results showed no staining in the test. This suggest the importance of callus line/genotype when developing the transformation system.

We have started changing the culture system to produce a more friable callus line similar to that seen . We initiated tests comparing agar and gelrite as well as levels of BA. Initial observations show that gelrite may have a significant effect on callus friability. We will continue observing this test.

We initiated a test to determine sensitivity of onion callus to glyphosate. Since we observed a low rate of transformation with a GUS construct it may be possible to obtain some transformation with glyphosate constructs. Selection should be possible with an appropriate glyphosate level, to be determined by this test.

none